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Technical Status

1.1.1 *Task 1 - Literature Review of Internal Microbial Corrosion*

Microbiologically influenced corrosion (MIC) is a complex and aggressive mode of corrosion [1-19]. A comprehensive literature review of publications, standard documents, research reports, and publications in scientific journals was conducted on the topic of internal MIC over a nine-month period. The literature review will be focused on information about MIC detection and limitation, MIC mitigation and prevention, and their relationship to overall pipeline corrosion, as well as those major factors or mechanisms which control the internal MIC process on metallic pipelines. The second focus of the literature review is to incorporate the data from Task 2 (conditions in raw biogas gathering line) and discuss its implications for potential microbial corrosion. The literature review will identify a set of major parameters for the construction of a preliminary MIC model in Task 3.

1.1.1.1 Background

Corrosion is mainly the consequence of electrochemical reactions on the surface of a metal. Its kinetics are determined by the physical/chemical environment at the metal surface, such as concentration of oxygen, salts, pH, reduction-oxidation (redox) potential, and conductivity (Figure 1). Microbiologically influenced corrosion (MIC) is corrosion influenced by the presence or activities of microorganisms including bacteria and fungi [20-23]. Microorganisms growing at the metal surface form a biofilm and the release of chemicals or the deposition of electrochemically active minerals from biofilms alters the rates and types of electrochemical reactions at the biofilm-metal surface interface and produces a broad range of outcomes such as pitting, crevice corrosion, under-deposit corrosion, selective dealloying, enhanced erosion and galvanic corrosion [22, 24-29] (Figure 2). The accurate diagnosis of MIC requires combination of microbiological, surface analytical and electrochemical techniques.

Figure 1. Internal Microbial Corrosion.
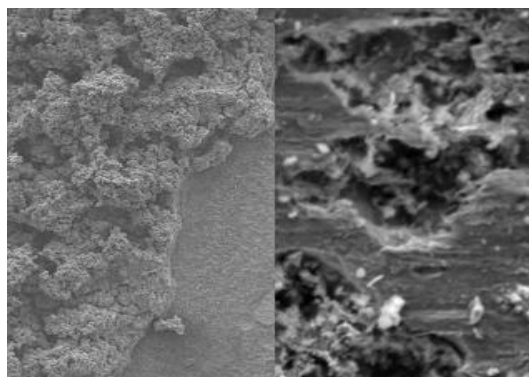
Figure 2. SEM Micrograph of Biofilm and Microbial Corrosion.

Despite the tremendous advances made in recent years to improve the knowledge and mechanisms of microbial corrosion, and development of better monitoring techniques, biocides, and other control measures, it is still not known with certainty how many species of microorganisms contribute to corrosion, how to reliably detect their presence prior to corrosion events, or how to rapidly assess the efficacy of mitigation procedures [2, 5-7, 23, 30-33].

MIC can occur in unexpected places. It tends to occur repeatedly at certain locations (Table 1) [34]. In general, MIC "problem areas" for many industries occur more often in welds and heat-affected zones, separators, drips, under film deposits, after hydrotesting, and when cooling systems are not passivated after "turnarounds" are complete.

1.1.1.2   MIC-Related Microorganisms and MIC Mechanisms

Many bacteria occurring naturally in waters and soils are considered corrosion-causing bacteria, including but not limited to, sulfate-reducing bacteria (SRB), acid-producing bacteria (APB), metal-oxidizing bacteria (MOB), metal-reducing bacteria (MRB), sulfur/sulfide oxidizing bacteria, nitrate-reducing bacteria, and slime-forming bacteria. Each of these physiological groups of microorganisms may contain hundreds or thousands of individual species. Each group of bacteria or an individual species of bacteria alone can cause metal corrosion; however in a natural environment, it is always microbial communities containing many different types of microbes that cause the MIC, and the resulting corrosion is always far more severe compared to the data generated under single strain laboratory conditions [3]. However, the mere presence of given classes of organisms associated with MIC (e.g., SRB) does not necessarily indicate that MIC is occurring. Nor does the showing that a given type of microorganisms is present establish a cause-and-effect relationship between the bacteria and metal dissolution [11, 35].

Many MIC mechanisms have been proposed since von Wolzgen Kuhr and Van Der Vlugt in 1934 [36]; most of them are focused on SRB corrosion [14, 17-19, 37-40]. A general mechanistic MIC model proposed by Pope includes three phases [28, 41] (Figure 3) . In Phase I, microbes attach to metal surface and start forming a biofilm. The attachment colonization of microbes in this phase is affected by many conditions such as preexisting corrosion on the metal surface, metal surface condition (roughness, welds, inclusions, etc.), and local chemical-electrochemical environments. The further development of biofilm on metal surface in Phase II creates an occluded area (inside and under the biofilms) that is relatively anodic to the surrounding area. In this phase, the occluded area becomes more acidic, attracting chloride and other anions and starts forming deposits on the metal surface (nodules or tubercles). Phase III involves the formation of a mature nodule over a well-defined pit. The low pH (<4.0) in the active pit region shifts the corrosion process to chemically-driven underdeposit acid attack. In this phase, the corrosion process would continue even in the absence of microbes [41].

Table 1. Where MIC is most likely to occur [34].

Industry/Application	Potential Problem Sites for MIC	Organisms Responsible
Pipelines-oil, gas, water, wastewater	Internal corrosion primarily at the bottom position Dead ends and stagnant areas Low points in long-distance pipes	Aerobic and anaerobic acid producers, SRB, manganese and iron-oxidizing bacteria, sulfur oxidizing bacteria
Chemical process industry	Heat exchangers, condensers, and storage tanks-especially at the bottom where there is sludge build-up Water distribution systems	Aerobic and anaerobic acid producers, SRB, manganese, and iron-oxidizing bacteria In oil storage tanks also methanogens, oil-hydrolyzing bacteria
Cooling water systems	Cooling towers Heat exchangers-in tubes and welded areas-on shell where water is on shell side	Algae, fungi, and other microorganisms in cooling towers Slime-forming bacteria, aerobic and anaerobic bacteria, metal-oxidizing bacteria, and other microorganisms and invertebrates
Fire protection systems	Dead ends and stagnant areas	Anaerobic bacteria, including SRB
Docks, piers, oil platforms, and other aquatic structures	Just below the low-tide line Splash zone	SRB below barnacles, mussels, and other areas sequestered from oxygen
Pulp and paper	Rotating cylinder machines Whitewater clarifiers	Slime-forming bacteria and fungi on paper-making machines Iron-oxidizing bacteria SRB in waste
Power generation plants	Heat exchangers and condensers Firewater distribution systems Intakes	As above for heat exchangers and fire protection systems Under mussels and other fouling organisms on intakes
Desalonation	Biofilm development on reverse osmosis membranes	Slime-forming bacteria

However, in a complex environment, a consortium of different types of microorganisms often work synergistically, resulting in far more severe corrosion compared to the data generated under single strain laboratory conditions [3]. For instance, APB produce low molecular weight organic acids (short chain fatty acids such as acetic, butyric, formic, lactic, succinic, and propionic acids) and inorganic acids (e.g., HCl, H_2CO_3 and H_2SO_4). While both types of acids can cause metal corrosion by either direct reaction with metal or disrupting the protective surface oxides films and calcium scales [11, 22, 42-47], the organic acids provide the environment and nutrients for the growth of other bacteria such as SRB [48] (Figure 4). In addition, biogenic acids increase the concentration of protons, which can then become reduced at the cathode, generating hydrogen, an electron source for SRB and other hydrogen-consuming organisms [11]. Activities of aerobic microbes deplete oxygen in the biofilm, create an environment for growth of anaerobic bacteria, and form an oxygen gradient within the biofilm. This causes a potential change beneath the film, resulting in the development of an anodic region surrounded by a large cathodic area and galvanic corrosion. In addition, if the protective oxide film is breached beneath a biofilm, then the metal cannot be reoxidize or self-heal. Oxygen gradients and breached oxide film result in metal pitting beneath biofilms. Therefore MIC is the consequence of collective effects of microbial consortia on metal surfaces.
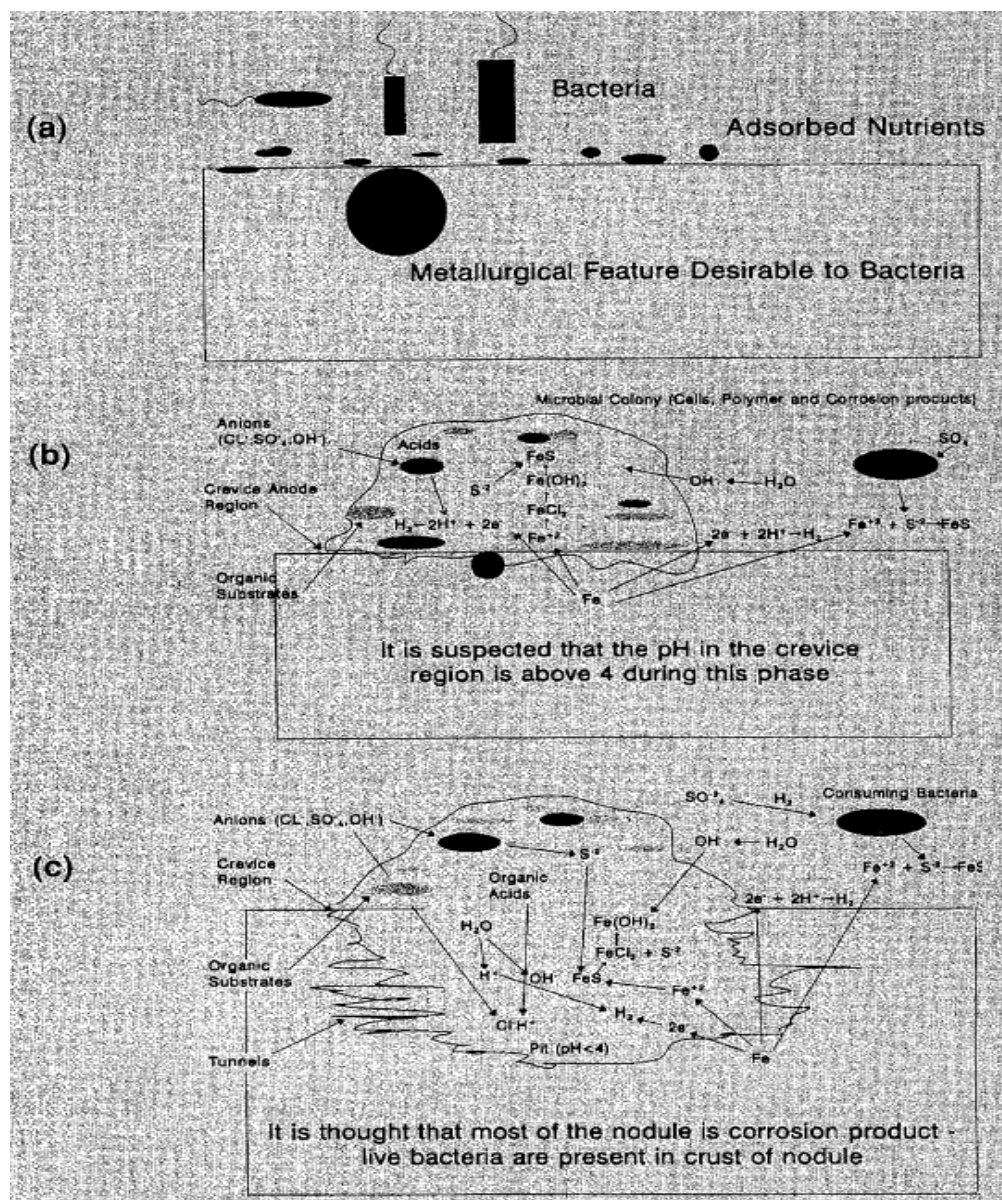
Figure 3. MIC Development Model [41]. (a) Recognition of Desirable Sites. (b) Colony Formation and Crevice Corrosion Begins and Anode is Fixed. (c) Nodule is Formed over "Mature" Pit.
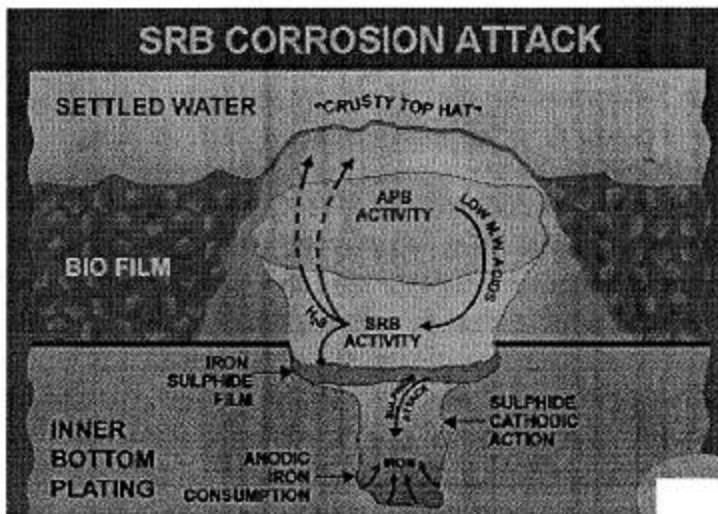
Figure 4. Interaction of SRB and APB on Metal Corrosion [49].

1.1.1.3 SRB-induced corrosion

Sulfate-reducing bacteria (SRB) constitute a physiologically diverse group of obligate anaerobic, heterotrophic, and mixotrophic bacteria that are responsible for dissimilatory sulfate reduction. They are present in a variety of environments, including oil- and gas-bearing formations, seawater, freshwater, soils, and domestic, industrial, and mining wastewaters [50]. Though SRB are anaerobic bacteria, SRB can survive and quickly recover from brief oxygen exposure [13, 39, 49]. SRB use hydrogen, organic acids (lactic, acetic, propionic, succinic, pyruvic, etc.), and variety of other low molecular weight organic compounds (ethanol, aliphatic acids, sugars, amino acids, indole, nicotinic acid, etc.) as electron donors and also as carbon and energy sources. Sulfate can be used as an electron acceptor for anaerobic respiration [51-53]. Previous microbiological studies have suggested that SRB play a key role in microbial corrosion [3, 30] and other problems of great economic impact in oil and gas industries [30]. For instance, oil reservoir souring is a well known phenomenon after seawater injection into reservoirs for oil extraction, i.e., the reservoir formation water provides volatile fatty acids (VFAs) as electron donors and the seawater provides the sulfate (~2,700 mg/L) as electron acceptor for SRB's anaerobic respiration.

It has been reported by many researchers that the corrosion rates caused by SRB under laboratory conditions are much lower than the rates under field conditions [10, 13-18, 38, 54], and the rates under laboratory conditions usually cannot be maintained at high level for long periods of time. The existence and activity of SRB causes the average corrosion rate of steel exposed to anaerobic soil to be more than 20 times higher than that of the control case, the maximum corrosion rate of steel and iron being reported by SRB to be 7.4 mm/y [14, 24, 55]. Pitting corrosion is characteristic of the action of SRBs on steel, with pits being open and filled with soft black corrosion products in the form of iron sulfides [10]. When the corrosion products are removed, the metal underneath is bright but rapidly rusts on exposure to air.

Various mechanisms have been proposed to explain the accelerated corrosion rate observed in the presence of SRB. The most classic among them is cathodic depolarization, proposed by

Von Wolzogen Kühr and Van Der Vlugt in 1934 [36]. They proposed that cathodic depolarization is achieved by the metabolic oxidation of hydrogen by SRBs.

$$4Fe \rightarrow 4Fe^{+2} + 8e^- \qquad \text{(anodic reaction)} \qquad (1)$$
$$8H_2O \rightarrow 8H^+ + 8OH^- \qquad \text{(water dissociation)} \qquad (2)$$
$$8H^+ + 8e^- \rightarrow 8H^0 \qquad \text{(cathodic reaction)} \qquad (3)$$
$$SO_4^{-2} + 8H^0 \text{ MIC} \rightarrow S^{-2} + 4H_2O \qquad \text{(cathodic depolarization)} \qquad (4)$$
$$Fe^{2+} + S^{-2} \rightarrow FeS \qquad \text{(corrosion products)} \qquad (5)$$
$$3Fe^{+2} + 6OH^- \rightarrow 3Fe(OH)_2 \qquad \text{(corrosion products)} \qquad (6)$$
$$4Fe + SO_4^{-2} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH^- \qquad \text{(overall reaction)} \qquad (7)$$
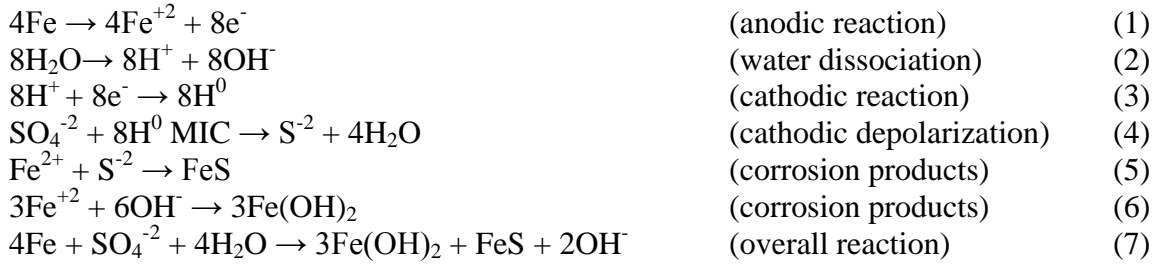
The cathodic depolarization theory posits that SRB at the cathode remove the H^0 from a polarized metal surface (through hydrogenase) for anaerobic respiration (to produce energy by reducing sulfate to sulfide), resulting in increased corrosion rate. However, many later researchers found evidence that conflicts with cathodic depolarization hypothesis [10]. It has been reported that the reactions occurring at the anode are at least as important as the cathode's and could be predominant in the case of SRB corrosion [56].

The most severe damage resulting from the corrosion of steel by SRB is most often localized, taking the form of pits, crates or similar clearly delimited areas of corrosion. Pitting corrosion is a process of the nucleation and growth type, and the mechanism of pitting corrosion is generally an autocatalytic stabilization of a galvanic cell between a small corroding area (the anode) and its non-corroding surroundings (the cathode). Thus, the more modern theory of SRB-induced corrosion involves the formation of ferrous sulfide film on metal surface and the formation of galvanic cell between ferrous sulfide film and steel base.

The galvanic corrosion theory states that under anaerobic conditions, SRB uses various electron donors (mainly small molecule organic acids) to reduce inorganic sulfate to sulfide. As a result, hydrogen sulfide accumulates in the biofilm near the metal surfaces and iron sulfide quickly forms on and covers the carbon steel surface. The iron sulfide film (cathode) and bare steel base forms a galvanic cell [57]. At the early stage, the film (mainly mackinawite, $FeS_{(1-x)}$, 35% S, dense and protective) is patchy and irregular, and therefore the SRB-induced corrosion rates are high due to the galvanic couple between the patchy iron sulfide (cathode) and the steel base (anode). However, after a uniform mackinawite film is formed, it protects metal from further corrosion, resulting in reduced SRB corrosion [24]. When mackinawite takes up more sulfide and gradually converts to greigite (Fe_3S_4) and pyrite (FeS_2, 52.5% S), the change in film density breaks the iron sulfide film and the resulting ruptured film exposes the bare metal, forms a galvanic corrosion cell again between the steel substrate and an unbroken sulfide film attached to the steel surface, and causes elevated corrosion rate [18, 58]. Pyrite is 12 times more corrosive than mackinawite due to higher potential difference to the iron anode (482 mV vs 610 mV). However, the incubation time for breakdown of mackinawite film dependant on various factors such as redox potential, solution chemistry, physical properties of films, is not predictable, and may take 2-3 months [14, 17]. A high concentration of ferrous iron in the medium may accelerate the breakdown of dense biogenic FeS film on the metal surface, and accelerate the corrosion rate [17, 19]. High amounts of soluble iron also prevent formation of protective sulfide

layers on ferrous metals [16]. Once mackinawite film is ruptured, the corrosion is independent of SRB number and growth rate.

The galvanic corrosion cell is normally short lived because the iron sulfide matrix becomes saturated with electrons derived from the corrosion process. However, anaerobic SRB remove electrons directly from FeS_x matrix (cathode), sustaining a flow of electrons through the galvanic couple from the corroding steel [18]. The microbes use these electrons to reduce sulfate to sulfide, which combines with ferrous ions (Fe^{2+}) derived from corrosion of the steel to precipitate more FeS_x, thus further increasing corrosive action.Other researchers found that the activity of the SRB on the anode (electrochemical or metabolic) might be more important than their activity on the cathode in terms of stabilizing the coupling current between the anode and the cathode, and proposed a theory that the SRB acidify the anode by precipitating ferrous ions into ferrous sulfide and stabilize the pH of the cathode, thus inducing a sustained galvanic coupling [56, 57, 59, 60]. The galvanic couple accounts for ~ 10% of the observed damage. Extension of the life of the corrosion cell through electron transfer to active bacteria is responsible for most of the metal loss [18]. Another classic hypothesis regarding the sustaining galvanic corrosion cell was proposed by King and Miller [17, 24, 61]. They attribute the sustaining life of galvanic cell to the adsorption of atomic hydrogen by the ferrous sulfide corrosion product. Ferrous sulfide is not, however, a permanent cathode [62] and its regeneration and the maintenance of a high sustained corrosion rate is dependent on the removal of this hydrogen by the action of bacterial hydrogenase.

Other alternative hypotheses also exist, and may contribute to SRB-induced corrosion. For instance, some SRB secrete exopolysaccharides (EPS), which facilitates irreversible cell attachment, leading to colonization on the steel surface. EPS can bind metal ions, causing metal ion concentration cells [63]. Hydrogen sulfide acidifies a corrosive medium and catalyzes penetration of hydrogen into steels, a process known as H_2S-induced cracking or sulfide stress cracking [64, 65]. Periodic oxygen incursions and sulfur/sulfide oxidizing bacteria can oxidize FeS_x to more corrosive sulfides such as pyrite (higher sulfur content) and production of elemental sulfur ($2S^{2-} + O_2 + 4H^+ -> 2S^{(0)} + 2H_2O$). Both products will increase corrosion significantly [13, 19, 39]. Elemental sulfur sustains the galvanic couple between iron and the corrosion product FeS_x by accepting electrons from the FeS_x. High local acidity generated on particles of solid sulfur reacting with water could also be responsible for high corrosion rates of iron and steel.

1.1.1.4 APB-induced corrosion

Acid-producing bacteria (APB) are present in a variety of environments, including oi1- and gas-bearing formations, soils, and domestic, industrial and mining wastewaters. Acid-producing bacteria produce organic acids (e.g., acetic, butyric, formic, lactic, succinic, and propionic acids) and inorganic acids (e.g., HCl, H_2CO_3, H_2SO_4), causing metal corrosion by either direct reaction with metal or disrupting the protective surface oxides films and calcium scales [11, 22, 42-47]. In addition, biogenic acids increase the concentration of protons, which can then become reduced at the cathode, generating hydrogen, an electron source for SRB and other hydrogen-consuming organisms [11, 57]. Short chain organic acids provide the nutrients for other bacteria growth such as SRB and can lead to general attack, pitting attack, and stress corrosion cracking [48]. Acetic

acid-producing bacteria and butyric acid-producing bacteria have been found to be present in environmental samples and in particular, samples from gas and oil production operations [4, 66, 67]. Consumption of hydrogen by SRB through formation of H_2S allows the APB to continue acid production. Some fungi also produce organic acids and other byproducts which support the growth of various other bacteria such as SRB [22].

1.1.1.5 MOB- induced corrosion

Metal-oxidizing bacteria (MOB), mainly iron-oxidizing bacteria and manganese-oxidizing bacteria, are generally filamentous, are typically found in fresh and marine water, and are frequently surrounded by a sheath usually encrusted with iron, manganese, or both. Iron-oxidizing bacteria such as *Gallionella*, *Sphaerotilus, Leptothrix, Siderocapsa, Thiobacillus, Crenothrix*, and *Clonothrix* oxidize the soluble ferrous (Fe^{2+}) and produce orange-red tubercles of iron oxides and hydroxides by oxidizing ferrous ions (electron donors) from the bulk medium or the substratum [68, 69]. They are commonly associated with tubercle formation and corrosion of water distribution pipelines. The small area under the deposit, deprived of oxygen, forms a galvanic cell with surrounding metal with large cathode to anode ratio, resulting in under-deposit corrosion, pitting, and crevice corrosion [22, 70], sometimes with assistance from sulfate-reducing bacteria [71]. *Gallionella* spp. contributes to the generation of conditions favorable to colonization by SRB [20]. Manganese-oxidizing bacteria oxidize the soluble manganese (Mn^{2+}) to insoluble manganese oxide (Mn_2O_3, $MnOOH$, Mn_3O_4, and MnO_2). The oxides are formed extracellularly and encrust the polymeric material (bacterial capsules) that surrounds individual cells or cell aggregates. *Leptothrix* and *Siderocapsa* are particularly associated with formation of highly enriched manganese oxide deposits. Manganese oxide can elevate corrosion current, and can also serve as a cathode to support corrosion at an oxygen depleted anode (metal surface) within the deposit, resulting in similar under-deposit corrosion, pitting, and crevice corrosion [22, 70].

The detection of iron- and manganese-oxidizing bacteria is usually dependent on diagnostic liquid cultures, which is very difficult even for experienced microbiologists. Microscopic identification of iron-oxidizing bacteria is also quite difficult for an experienced analyst. Several direct and indirect tests for the presence of corrosion-causing bacteria are summarized in NACE Standard TM0101-2006 [22]. However, these techniques are not capable of quantifying metal-oxidizing bacteria. A new technique called quantitative polymerase chain reaction (qPCR) is now available for quick detection and quantification by targeting 16S rRNA gene of *Leptothrix*, *Sphaerotilus*, and *Gallionella* [72, 73]. The presence of iron-oxidizing bacteria within tubercles associated with localized corrosion is considered a positive indication of MIC.

1.1.1.6 MRB-induced corrosion

Under oxic conditions, the metal surface becomes oxidized, causing the formation of metal oxides and hydroxides, which protect the metal surface from further corrosion. Some metal-reducing bacteria (MRB) are capable of using metal oxides or hydroxides (Fe^{3+} and Mn^{4+}) as electron acceptors efficiently (i.e., redox potential is similar to nitrate) and out-compete low potential electron acceptors such as sulfate or carbon dioxide [74]. When MRB is in direct contact with solid iron (Fe^{3+}) and manganese (Mn^{4+}) oxides, the dissimilatory reduction produces soluble ions (Fe^{2+} and Mn^{2+}), resulting in dissolution of surface oxides. This destabilizes the passivating protective film (oxide film) and allows further corrosion (localized corrosion) to take

place [11, 48]. Medium containing ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$) as the terminal electron acceptor and acetate as the sole carbon source can be used to detect the presence of IOB. A positive indication of growth and iron reduction is a color change in the medium from brown to green [22].

1.1.1.7   Other bacteria-induced corrosion

Acidophilic sulfur/sulfide-oxidizing bacteria oxidize sulfide or elemental sulfur to sulfate or sulfuric acid. For example, *Thiobacillus* bacteria are the most common sulfur-oxidizing bacteria, and are almost always accompanied by SRB. Sulfur/sulfide-oxidizing bacteria obtain the carbon required for the synthesis of new cell material by fixation of CO_2 from the atmosphere and energy from oxidation and reduction reactions [64, 75]. Ferrous iron from reduced sulfur compounds serve as the electron donor, and oxygen is the preferred electron acceptor. In the absence of oxygen, organisms grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor. The specific oxidation reactions leading to production of sulfuric acid (H_2SO_4) varies with the initial reduced sulfur species (H_2S, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$, S^0). Elemental sulfur, thiosulfates, metal sulfides, H_2S, and tetrathionates can be oxidized to H_2SO_4 [76].

Methanogens and some strains of SRB frequently co-exist in a symbiotic relationship. They remove hydrogen from the surface of metals catalyzed by a reversible hydrogenase, enhance the cathodic reduction of proton (cathodic depolarization), and thereby accelerate anodic metal dissolution [11, 77]. Culturing of methanogens is very difficult due to the strictly anaerobic nature of methanogens. A genetic technique is now available for quick detection and quantification of methanogens by targeting a specific functional gene [72].

Nitrate- and nitrite-reducing bacteria use nitrogen oxides as alternative electron acceptors under anoxic conditions [78]. In the presence of nitrate, denitrifying bacteria are reported to cause metal corrosion [31, 79].

Hydrogen embrittlement of metals occurs when molecular hydrogen invades the metal lattice, filling interstitial regions and thereby distorting the lattice structure and weakening the metal-metal bond [11]. Bacterial production of hydrogen can directly promote hydrogen embrittlement of metals. Indirectly, the generation of acids, which can be reduced to hydrogen at cathodic sites, as well as the generation of sulfide (which promotes the adsorption of hydrogen into metal matrices) may also promote hydrogen embrittlement.

1.1.1.8   MIC Detection and Monitoring

Internal MIC is a significant problem affecting the oil and gas and other industries. Routine monitoring of water quality may identify potential problem organisms and the factors that may promote bacterial growth and attack. Water quality parameters that are considered important to understanding internal corrosion and MIC for a particular industrial system include temperature, pH, alkalinity, sulfide, nitrite, dissolved gases (CO_2, H_2S, O_2, NH_3, etc.), total dissolved solid (TDS), chemical oxygen demand (COD), microorganisms (bacteria, algae, and fungi), etc. COD measures the concentration of electron donors available for sulfate or metal reduction; hence a low COD means a low risk of finding SRB and iron-reducing bacteria in the system. On the other hand, dissolved oxygen might not be indicative as to the oxygen content within the biofilm.

Nevertheless, changes in these parameters, especially long-term trends in one direction or large anomalies, indicate a need for further investigation. Online monitors are commercially available for monitoring temperature, pH, conductivity, and TDS, and portable or laboratory spectrophotometers and kits are available for the other tests. MIC investigations require microbiological, chemical, and metallurgical testing for proper diagnosis.

Free-floating planktonic bacteria are often the focus of monitoring for MIC since system fluids are generally easier to sample than metallic surface. However, the results of planktonic bacteria can sometimes be misleading as to whether MIC will occur or, if so, to what extent [80, 81]. Many bacteria such as *Pseudomonas*, *Serratia*, and SRB secrete EPS, which improves the adherence capacity to a metal surface and promotes further trapping of microorganisms in the substratum. The environmental conditions at biofilm/surface interfaces are often radically different from the bulk medium in terms of pH, dissolved oxygen, and other organic and inorganic species. Oxygen consumption by aerobic bacteria living in the surface region of the biofilm leads to the creation of an anaerobic space for the growth of anaerobic bacteria, which, in turn, results in the formation of oxygen concentration gradients and differential aeration cell on a metal surface [60]. The most devastating MIC takes place in the presence of microbial consortia in which many physiological types of bacteria, including SRB, APB, MOB and MRB, interact in a complex way within the structure of biofilms [3, 54, 82]. Compared to planktonic bacterial counts, sessile bacteria (e.g., biofilm) are more relevant to microbial corrosion [83]. However, monitoring sessile bacteria or biofilm is more complicated, requiring either that the pipeline be excavated or halted for internal sampling or that accommodations be made in the system design to allow for regular collection or on-line tracking of attached organisms during operation.

The most commonly used means of monitoring MIC is to quantify the number of bacteria capable of growing in various microbial growth media (solid or liquid) after inoculation with water samples (serial dilution) obtained from pipelines and other locations [81, 84]. Solid samples such as internal deposits, corrosion products, and surface swabs should be suspended in a sterile phosphate buffer to release viable microbes for inoculation. After incubation at certain temperature for a pre-determined period of time (days to weeks), the result is expressed as the number of colony forming units (CFU) for solid medium or the most probable number (MPN) for liquid medium. Many bacteria growth media are commercially available or can be made in the laboratory to selectively grow and detect certain type of microbes – aerobic bacteria, anaerobic bacteria, APB, SRB, sulfur-oxidizing bacteria, iron-related bacteria, low nutrient bacteria, nitrite/nitrate-reducing bacteria, and slime-forming bacteria, fungi, algae, etc. General aerobic or anaerobic bacteria counts are normally always included in a MIC monitoring program to gauge the environmental conditions for microbial growth. Some microorganisms such as sulfur-oxidizing bacteria, iron-oxidizing bacteria, and iron-reducing bacteria are very difficult to grow in culture, and the indicators for active growth sometimes are not always appropriate or easy to identify. It is also important to note that the bacterial growth media that are intended to support the growth of a particular type of bacteria are not completely selective, and the vast majority (90-99%) of microbial species cannot currently be grown in the laboratory [85-88], thus underestimating the size and misrepresenting the true composition of microbial communities in the sample [73, 89, 90].

Correct and consistent procedures are crucial for the success of growth methods in MIC monitoring. Sample collection may expose microorganisms to abrupt changes in pressure, temperature, atmosphere, and light, causing redistribution in numbers and types of microorganisms in the original samples. Therefore, the sample collection method, sample transportation, culturing techniques and growth medium, incubation temperature and time should be strictly controlled in order to reveal trends in bacteria number over long periods of time. This information is far more important and useful than a single data point when detecting and monitoring microbial corrosion in a particular system. NACE Standard TM0194-2004 details the sampling procedures for planktonic bacteria, culturing techniques, growth medium and growth indicator for general heterotrophic bacteria and SRB, and provides the guidelines for the assessment of sessile bacteria [81].

To circumvent problems associated with cultivation-based methods, many culture-independent genetic techniques have been developed in the past decade [91, 92], and are beginning to be used in the oil and gas industry for problems related to MIC. One such method is called reverse sample genome probing (RSGP), which allows determination of up to 30 SRB species on an environmental sample in a single DNA hybridization assay [93-95]. Another example of genetic method is quantitative polymerase chain reaction (qPCR) [72, 96-98]. qPCR can be designed to target and quantify a specific gene which only exists in a specific species or specific group of bacteria, such as SRB, APB and IOB. qPCR has also been used to determine microorganism abundance in many different types of complex environmental samples such as sediments, water, wastewater, feces, and marine samples, from domain down to genus and species levels [97-101]. The results are more accurate and can be obtained in a few hours instead of days or weeks required for traditional growth methods [72, 73]. Unlike traditional culturing method, qPCR detects and quantifies the target microorganisms in the samples without cultivation, thus it does not alter the composition of the microbial community in the original sample. In addition, qPCR also works for dry and old samples without live bacteria, a huge advantage over traditional growth methods.

Bacteria in the water sample can also be directly counted under a microscope with or without staining. With proper staining (e.g., fluorescent dye), it is even possible to distinguish the live and dead bacteria under a microscope. If bacteria are stained with fluorescently labeled oligonucleotides, it is possible to identify the genera or species of microbes in microbial communities, helping understand how biofilms develop and influence corrosion processes. However, direct counting with a microscope is difficult, time consuming and sometimes impossible when the sample is turbid or colored, and requires a well-trained observer to gain useful information. Hydrocarbon, deposits, and other contaminants in the sample occasionally fluoresce under ultraviolet light thereby preventing the use of fluorescent dye. Other enumeration methods involve the measurement of molecules peculiar to microbes (e.g., antibody-based SRB enumeration), or biochemical activities (e.g., hydrogenase-based SRB enumeration, adenosine triphosphate or ATP assay). These methods are generally difficult to calibrate against "real world" microbes and have high detection limits.

Chemical characterization of corrosion products and bulk fluids collected from corrosion sites is also important in the diagnosis of MIC. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), ion chromatography (IC), and other traditional colorimetric and

spectrophotometric assays are commonly used to measure elemental concentrations in water or pipeline deposit samples. Metallurgical testing techniques include energy-dispersive x-ray spectroscopy (EDX), x-ray diffraction (XRD) analysis and Raman spectroscopy. These are used to analyze corrosion morphology (pitting depth, shape, coverage, etc.) and corrosion products (chemical composition, compounds, etc.). Other techniques such as scanning electron microscope (SEM), environmental SEM (ESEM), and confocal scanning laser microscope can also be used to qualitatively evaluate the biofilm and/or corrosion products [11]. The integrated consideration of chemical and metallurgical data, microbial data and operational conditions is needed for proper detection and diagnosis of MIC [11].

The choice of internal corrosion (including MIC) monitoring is based on variety of factors, such as leak history, product quality, presence of corrosion indicators detected in previous samples (e.g., dew point and/or free water levels, acid gas pressures, iron, and bacteria counts, etc.), as well as other operational and economic factors. In many oil and gas operations, monitoring has often combined with the use of corrosion detection devices with sampling and analysis of gas, liquids, and solids obtained from the system. Under some conditions, microbial corrosion and overall internal corrosion may be monitored using corrosion coupons or probes. The coupons are made from an alloy similar to the metal in the system, and typically installed in the bottom quadrant of gas lines so they would be exposed to any liquids that condensed or are inadvertently put into the system, or in a "side-stream" which offers the additional advantage of allowing one to experimentally alter biocide levels and process conditions, giving reasonably fast and reliable information on their affects on the system. The presence of biofilm and microbial activities on a coupon surface change the local chemistry, possibly modifying the local anodic and cathodic processes and initiating or dramatically altering corrosion process such as pitting. Extensive microscopic analysis of coupons can yield important evidence with regard to pit initiation mechanisms, identify the severity of localized attack through the measurement of pitting (pit densities, depths, and diameters), calculate pitting rates by bacteria or other corrosive components, and determine the severity of attack.

The drawback of corrosion (including MIC) monitoring with metal coupons or probes is that it is destructive and requires time-consuming analysis of numerous coupons sequentially placed in the pipeline in order to obtain information on long-term buildup of biofilms and corrosion initiation. Various electrochemical techniques have been developed for nondestructive and long-term monitoring of the formation and activity of biofilm and possibly detection of an early MIC problem [83, 102, 103]. Such electrochemical techniques include electrical resistance (ER) probes, linear polarization resistance (LPR) probes, galvanic probes, hydrogen probes, electrochemical impedance spectroscopy (EIS), electrochemical noise (ECN), etc. ER probes are used to determine metal loss by measuring the increase in resistance of a metal specimen as its cross-sectional area is reduced by corrosion. LPR probes measure instantaneous corrosion rates and qualitative pitting tendency of metals in electrolytes. ECN measures the fluctuations of the potential, current and resistance over time and then determines the overall corrosion rates and rapid sustained pitting (RSP). For example, Hernández-Gayosso and colleagues successfully detected the formation of biofilm, increased corrosion rate and initiation of localized corrosion on electrodes using EIS technology [83].

One drawback to most electrochemical techniques is the need for electrolytes in the area of the measuring device. Another weakness of most electrochemical techniques is the failure to quantify the localized corrosion, especially RSP [104, 105]. These techniques give average readings for the surface of a test electrode, and it is not clear whether a measured corrosion current corresponds to uniform corrosion of the entire surface or to localized corrosion of just a few sites on the surface. In the latter case, corrosion rates will be severely underestimated if the measured corrosion loss is not normalized to the area at which localized corrosion occurs. This general disadvantage of electrochemical techniques is especially bothersome in the case of MIC, where most corrosion processes are of an extremely localized nature [11, 48].

1.1.1.9   MIC Prevention and Mitigation

Once internal MIC has been established in a pipeline, complete mitigation is neither practical nor possible. Therefore, the prevention of internal MIC from being initially established should be a top priority. One of the first defense systems against internal corrosion is to ensure that the product being transported is free of moisture. For corrosion to occur, there must be moisture, CO_2, O_2, or some other reduction reactant, such as one produced by microbes. Gathering lines in production fields have a much more significant problem with internal corrosion than the typical transmission pipeline. MIC after hydrotesting is a common problem when the system was not completely dried after testing. Water used in hydrotesting should be as clean as possible by removing particulates, contaminants and nutrients such as oils, iron, phosphate, and nitrate. When necessary, water should be treated to reduce hardness, remove oxygen, or alter pH.

Although coatings/linings have been used on the internal aspects of natural gas pipelines principally to improve flow characteristics, some internal linings also appear to protect against at least some forms of corrosion, including MIC, by effectively isolating the pipeline from the impact of surrounding environment [106]. However, due to its feasibility and cost, internal coatings are generally limited to new installations or areas easily accessible to "in situ" lining and areas in which pigging would not destroy the integrity of the lining. It should be noted that the target area must be completely lined. Failure to coat weld regions or other features in contact with lined portions of the system could focus corrosion on the unlined areas, thereby accelerating corrosion in these areas. In addition, coating performance can be compromised by microbial degradation of coatings or components in the coating system, leading to water permeation and disbondment of coating. MIC regularly takes place on pipe surfaces under the disbonded coatings, where water and nutrients promote the growth of microorganisms, resulting in the formation of a corrosion cell. The severity of corrosion under the disbonded coating strongly depends on the conductivity of the water trapped in the pocket under the separated coating.

System design, maintenance, and water quality are the keys to MIC prevention and control [34, 107]. Materials selection, accessibility for cleaning and water treatment, provision for drains, traps, recycle circuits, and monitoring equipment, control of water velocity and elimination of stagnant, low-flow areas and dead legs, and minimization of crevices and welds are the key considerations in system design. Regular cleaning, including chemical and mechanical cleaning, should be part of the operating routine to remove sludge, deposits, and foulants from the system.

The mitigation measures of internal MIC consist primarily of mechanical cleaning (pigging) and chemical treatment (biocides and corrosion inhibitors). Chemical treatments usually involve the use (in batch or continuously) of biocides, corrosion inhibitors or both to control microbes in the system. A successful MIC control program requires assessment of the MIC potential in a system, screening tests of chemical treatments, and aggressive monitoring of actual systems after treatment. It is worth noting that most laboratory studies of biocide efficiency in man-made system often fail to duplicate their successful results when they are applied in industrial systems. Organisms embedded within the biofilm are protected from biocides, largely due to the diffusion barriers generated by the EPS matrix that hinders the chemical penetration of the entire thickness of the deposits [23, 108]. Moreover, bacteria within the biofilm are probably physiologically altered and may develop resistance to a particular biocide if it is used repeatedly [2, 109]. Therefore, before the biocide treatment, a "time-kill" study is often needed to identify what chemical agent(s) are the most effective in killing the bacteria in a particular system.

The resistance of bacteria to biocides depends on the nature of the chemicals used. Biocides can be classified as either oxidizing or non-oxidizing. Apart from ozone and hydrogen peroxide, all the oxidizing agents used as biocides contain halogens. The non-oxidizers are relatively non-reactive chemicals and, therefore, compatible with strong reducing agents in water treatment application [110]. Examples of typical non-oxidizing biocides are formaldehyde, glutaraldehyde, methanol, isothiazolones, quaternary amines, and tetrakishydroxymethylphosphonium sulfate (THPS). Non-oxidizing biocides are often used in combination with dispersants and surfactants to stimulate full biocides penetration into the biofilm. Whether biocides can be used continuously or in a batch mode, or periodically, depends on the system. In the case of continuous treatment, it is necessary to alternate several biocides to prevent biocide resistant bacteria strain from being developed. Batch treatment is usually applied to the system after hydrotesting and pigging operation. The effectiveness of biocide treatments depend on proper treatment schedule, effective doses, and appropriate locations, and combination with other control technologies (e.g., pigging) [106]. For instance, an additional pigging run using a sphere or ball pig to push a slug of a biocide solution (1% cocodiamine and quaternary in methanol) was reported to be very effective to keep the pipe free of bacteria after hydrotesting [2]. The mixture biocide solution in this treatment also acts as a corrosion inhibitor against carbon dioxide and hydrogen sulfide attack.

Batch or continuous injection of corrosion inhibitors is also commonly employed to treat/prevent many types of corrosion including MIC. Most corrosion inhibitors used in the natural gas industry are more effective in preventing and treating generalized-type corrosion than the focused, RSP corrosion usually associated with MIC, due to the difficulty in penetrating existing biofilms and corrosion products and to the fact that bacteria may degrade some corrosion inhibitors [4, 111]. The concentrations of biocides and corrosion inhibitors have to be closely monitored in the system during treatment since the treatment chemicals can be degraded or used up faster by factors such as pH, TDS, chlorides, temperature, oxygen, etc. Spore-forming microorganisms such as species in genus *Bacillus* and *Clostridium* can usually survive biocide treatment, and re-generate in the pipeline system when biocide concentration becomes lower and other conditions become favorable. *Bacillus* has been isolated frequently from tubercles formed on metals and associated with microfouling [20]. These organisms are copious producers of organic acids.

"Pigs" are the most common device used for the mechanical cleaning of the pipeline interior, and pigging is one of the most effective means of controlling microbes on metal surfaces and, therefore, internal MIC pigs are inserted into the pipelines and pushed through the pipe using gas pressure. The frequency of pigging and types of pigs utilized are determined, at least in part, by the results of the pigging itself, such as the amount and types of materials removed from the line. The objectives of mechanical cleaning are to remove materials capable of inhibiting gas flow and/or promoting corrosion (including MIC) from the pipeline. These materials include fluids (including water) and solids (e.g., sand, corrosion products, nodules, and biofilms/slimes). Water is required for microbial metabolism and growth and corrosion processes, reduces the efficiency of treatment chemicals (e.g., biocides and corrosion inhibitors), and allows the formation of concentration cells. Solids provide shelter for microorganisms and water.

In addition to viable microbes in the removed materials, pH, iron, chloride, and sulfide should also be measured in the monitoring program. Chloride (Cl^-) ions are very aggressive and participate in many forms of corrosion, including MIC. Chloride ions from the electrolyte migrate to the anode to neutralize any buildup of charge, forming heavy metal chlorides that are extremely corrosive to metal surface, particularly stainless steels. Under these circumstances, pitting involves the conventional features of differential aeration, a large cathode-to- anode surface area, and the development of acidity and metallic chlorides [22]. Webster and Newman examined the impact of media constituents on localized corrosion and concluded that localized corrosion would not readily occur unless chloride ion was the predominant anion in the medium [112]. Sulfide levels in the corrosion products and fluids can serve as an indication of MIC-type corrosion.

A very different approach which has been proposed as a potential alternative to protect pipeline from internal corrosion is to use beneficial biofilm on metal surface as a corrosion inhibition mechanism [113]. Biofilms have been reported to be effective on inhibition of general corrosion in some circumstance for mild steel, copper, aluminum, and stainless steels [114-119]. The mechanisms most frequently cited for the inhibition are
1) formation of a diffusion barrier to corrosion products that stifles metal dissolution,
2) removal of corrosive agents (e.g. oxygen) from metal surface by bacteria physiological activities (e.g. aerobic respiration) [120, 121],
3) growth inhibition of corrosion-causing bacteria by antimicrobials generated within biofilm (e.g., SRB corrosion inhibition by gramicidin S-producing *Bacillus brevis* biofilm [115, 117, 118],
4) generation of protective layer by biofilms (e.g., *Bacillus licheniformis* biofilm produces on aluminum surface a sticky protective layer of gamma-polyglutamate) [120],
5) formation of passive layers (e.g. magnetite film) [120], and
6) production of metabolic products that act as corrosion inhibitors (e.g., siderophores) [122, 123].

However, biofilm formation on metal surface is unpredictable and uncontrollable, and is often not uniform. Bacteria tend to colonize preferentially on rough surfaces and are more attracted to anodic sites [124]. Biofilm growth rate depends on substratum, available nutrients, temperature, and electron acceptors. Biofilm composition is affected by small

perturbations in the environment (e.g., temperature, nutrient concentration, and flow). A little understood phenomenon – biofilm sloughing – creates a discontinuity of biofilm on metal surface (patchiness), which results in local differences in metabolic products, pH, dissolved oxygen, and gradients of nutrients and ions within the biofilm. Patchy biofilms create differential aeration cells which can lead to intensification of localized corrosion rates under the biofilms [125, 126]. Biofilm formation is an extremely complex biological/chemical process, and its impact on corrosion processes is difficult to predict and control. Therefore, more research is needed before biofilms can be used as corrosion inhibition mechanisms in the field.

1.1.2 *Task 1 - Collection of biogas/biomethane data (Chemical)*

The center piece of this research is the need to understand the integrity impacts of transporting various biogas/biomethane products through existing non-metallic pipelines and its non-metallic components. To aid in this investigation it is necessary to fully comprehend the composition of potential fuel gases that may come into contact with these materials.

One phase of task one includes the collection of analytical data from biogas and biomethane derived from dairy manure, landfills, and wastewater treatment plants (WWTP). Data was obtained from previous GTI projects as well as samples sent to the analytical laboratory from industrial customers. In addition, an extensive literature review was performed and several datasets from biogas facilities were obtained. This data will be compared with comprehensive natural gas data sets acquired over the past two decades.

Consistent with GTI's previous research projects, constituents of the biogas or biomethane were categorized into two tiers. The *First Tier* includes compounds that are consistent with those found in natural gas pipeline tariffs.

Table 2and Table 3 include target compounds from the First Tier grouped based on analytical methods used for detection and quantification. *Second Tier* target compounds include compounds of concern which are not routinely monitored in natural gas but are a potential hazard to both human health and infrastructure. Table 4 and Table 5 include target compounds from the Second Tier and is also grouped based on analytical methods used for detection and quantification. Not all samples were subject to all analyses. Data collected from literature review and samples from industrial customers provided data for biogas of only the First Tier compound list; however, only a partial list of compounds was acquired for some samples.

GTI project *Pipeline Quality Biomethane: North American Guidance Document for Introduction of Dairy Waste Derived Biomethane into Existing Natural Gas Networks* evaluated biogas and biomethane from dairy farms from three geographic regions in the United States. Samples from 14 different farms producing biogas and/or biomethane were collected and analyzed for all chemical groups in the first and second tier. Twelve samples of raw biogas were collected from 12 different farms. Another 7 samples were collected from 5 biogas facilities that provided partial upgrading. A total of 23 samples from 2 biogas facilities that executed full upgrading to biomethane were collected. The data from these samples are attached as Appendix A.

As part of GTI's *Pipeline Quality Biogas: Guidance Document for Dairy Waste, Wastewater Treatment Sludge and Landfill Conversion* (PHMSA Project 250), a total of 47 samples were analyzed for all compound groups in the first and second tier. This includes 16 biomethane samples from landfill gas and 5 biomethane samples from WWTPs, 14 biogas samples from landfill and 5 from WWTPs, and 7 natural gas samples. Results from these samples are provided in Appendix B.

GTI's analytical laboratory provides a variety of analytical services for the natural gas and fuels-related industry. Many industrial customers send samples to GTI for analysis of biogas. In the past four years, a total of 346 biogas samples were analyzed by the laboratory for first tier chemical groups; 41 from dairy farms, 170 from landfills, and 135 from wastewater treatment facilities. In addition, 20 natural gas trace constituent samples were obtained. A list of the samples and chemical information available is provided in Appendix C.

Previous GTI (formerly GRI and IGT) projects evaluated trace constituents in natural gas from across the United States and Canada. The projects provide analytical data from the first tier chemical group. A total of 31 samples were obtained for review for this project. Analytical data for natural gas samples from these projects and the PHMSA project will be compiled into table format and presented in the next quarterly update.

A literature review of existing analytical data from biogas and biomethane yielded 37 samples from 4 reports; 21 biogas samples from landfill, 2 from partially cleaned biogas from landfill, 6 biogas samples from dairy and 8 biogas samples from WWTP. However, data from these reports present data only on major components of biogas and some data on volatile organic compounds (VOCs). Data from these reports have not been compiled into table format.

Table 5 summarizes the number of samples collected for review based on type of gas and source of information. During the next quarter, the data from all sources will be organized into a consistent table format for easier review.

Table 2. Target Compounds for First Tier Chemical Testing, Part A

Halocarbons	Siloxanes	Metals
Dichlorodifluoromethane (CFC-12)	1,1,3,3-Tetramethyldisiloxane	Arsenic
1,2-Dichlorotetrafluoroethane (CFC-114)	Pentamethyldisiloxane	Barium
1,1,2-Trichloro-1,2,2-trifluoroethane (CFC-113)	Hexamethyldisilane	Beryllium
Trichlorofluoromethane (CFC-11)	Hexamethyldisiloxane	Cadmium
Chloromethane	Octamethyltrisiloxane	Cobalt
Dichloromethane (Methylene Chloride)	Octamethylcyclotetrasiloxane	Chromium
Chloroform	Decamethyltetrasiloxane	Copper
Carbon Tetrachloride	Decamethylcyclopentasiloxane	Manganese
Chloroethane	Dodecamethylpentasiloxane	Molybdenum
1,1-Dichloroethane		Nickel
1,2-Dichloroethane		Lead
1,1,1-Trichloroethane		Antinomy
1,1,2-Trichloroethane		Selenium
1,1,2,2-Tetrachloroethane		Strontium
Chloroethene (Vinyl Chloride)		Thallium
1,1-Dichloroethene		Zinc
cis-1,2-Dichloroethene		
Trichloroethene		
Tetrachloroethene		
1,2-Dichloropropane		
3-Chloropropene		
cis-1,3-Dichloropropene		
trans-1,3-Dichloropropene		
Bromomethane		
1,2-Dibromoethane		
Chlorobenzene		
1,2-Dichlorobenzene		
1,3-Dichlorobenzene		

1,4-Dichlorobenzene			
1,2,4-Trichlorobenzene			
Hexachloro-1,3-butadiene			

Table 3. Target Compounds for First Tier Chemical Testing, Part B

Major Components	Extended Hydrocarbons	Sulfur Compounds	Calculated Real Gas Properties
Helium	Cyclopentane	Hydrogen Sulfide	Compressibility Factor
Hydrogen	Methylcyclopentane	Sulfur Dioxide	Specific Gravity
Carbon Dioxide	Cyclohexane	Carbonyl Sulfide	Gross HV (Btu/ft^3)
Oxygen/Argon	Methylcyclohexane	Carbon Disulfide	Wobbe Index
Nitrogen	Benzene	Methyl Mercaptan	Net HV (Btu/ft^3)
Carbon Monoxide	Toluene	Ethyl Mercaptan	Density
Methane	Ethylbenzene	i-Propyl Mercaptan	
Ethane	m,p-Xylene	n-Propyl Mercaptan	
Ethene	Styrene	t-Butyl Mercaptan	
Ethyne	o-Xylene	Dimethyl Sulfide	
Propane	C3 Benzenes	Methyl Ethyl Sulfide	
Propene	Naphthalene	Diethyl Sulfide	
Propadiene	C1 Naphthalenes	Di-t-Butyl Sulfide	
Propyne	C2 Naphthalenes	Dimethyl Disulfide	
i-Butane	Hexanes	Methyl Ethyl Disulfide	
n-Butane	Heptanes	Methyl i-Propyl Disulfide	
1-Butene	2,2,4-Trimethylpentane	Diethyl Disulfide	
i-Butene	Octanes	Methyl n-Propyl Disulfide	
trans-2-Butene	Nonanes	Methyl t-Butyl Disulfide	
cis-2-Butene	Decanes	Ethyl i-Propyl Disulfide	
1,3-Butadiene	Undecanes	Ethyl n-Propyl Disulfide	
i-Pentane	Dodecanes	Ethyl t-Butyl Disulfide	
n-Pentane	Tridecanes	Di-i-Propyl Disulfide	
neo-Pentane	Tetradecanes	i-Propyl n-Propyl Disulfide	
Pentenes	Pentadecanes	Di-n-Propyl Disulfide	
Hexane Plus	Hexadecanes	i-Propyl t-Butyl Disulfide	
Ammonia	Heptadecanes	n-Propyl t-Butyl Disulfide	
	Octadecanes	Di-t-Butyl Disulfide	
	Nonadecanes	Dimethyl Trisulfide	
	Eicosanes +	Diethyl Trisulfide	
		Di-t-Butyl Trisulfide	
		Thiophene	
		C1-Thiophenes	
		C2-Thiophenes	
		C3-Thiophenes	
		Benzothiophene	
		C1-Benzothiophenes	
		C2-Benzothiophenes	
		Thiophane	
		Thiophenol	

Table 4. Target Compounds for Second Tier Chemical Testing, Part A

Semi-volatile/Volatile Organic Compounds			Aldehydes/ Ketones
1,1,1-Trichloroethane	2-Chlorophenol	2,6-dinitrotoluene	Formaldehyde
1,2-Dichloroethane	1,3-Dichlorobenzene	1,2-Dinitrobenzene	Acetaldehyde
1,1-Dichloropropene	1,4-Dichlorobenzene	3-Nitroaniline	o-Tolualdehyde
Benzene	p-Isopropyltoluene	Acenaphthene	Acetone
Carbon Tetrachloride	Benzyl Alcohol	2,4-Dinitrophenol	Isocaleraldehyde
1,2-Dichloropropane	2-Methylphenol (m-cresol)	4-Nitrophenol	Valeraldehyde
Trichloroethene	1,2-Dichlorobenzene	Dibenzofuran	Butyraldehyde
Dibromomethane	3,4-Methylphenol	2,4-dinitrotoluene	m-Tolualdehyde
Bromodichloromethane	bis(2-chloroisopropyl)ether	2,3,4,6-Tetrachlorophenol	Propionaldehyde
Pyridine	n-Butylbenzene	2,3,5,6-Tetrachlorophenol	Crotonaldehyde
cis-1,3-Dichloropropene	N-nitroso-di-n-propylamine	Diethylphthalate	2,5-Dimethyl-
N-nitrosodimethylamine	Hexachloroethane	4-Chlorophenyl-phenylether	benzaldehyde
Toluene	1,2-Dibromo-3-	Fluorene	Benzaldehyde
trans-1,3-Dichloropropene	Chloropropane	4-Nitroaniline	p-Tolualdehyde
1,1,2-Trichloroethane	Nitrobenzene	4,6-Dinitro-2-methylphenol	Hexanaldehdye
1,3-Dichloropropane	Isophorone	n-Nitrosodiphenylamine	Methyl ethyl
Dibromochloromethane	2-Nitrophenol	4-Bromophenyl phenyl	ketone
1,2-Dibromoethane	2,4-Dimethylphenol	ether	
Tetrachloroethene	bis(2-Chloroethoxy)	Hexachlorobenzene	
Chlorobenzene	methane	Pentachlorophenol	
1,1,1,2-Tetrachloroethane	1,2,4-Trichlorobenzene	Phenanthrene	
Ethylbenzene	Naphthalene	Anthracene	
m/p-Xylenes	2,4-Dichlorophenol	Carbazole	
Bromoform	4-Chloroaniline	Di-n-butylphthalate	
Styrene	Hexachlorobutadiene	Bis(2-ethylhexyl) adipate	
o-Xylene	1,2,3-Trichlorobenzene	Fluoranthene	
1,1,2,2-Tetrachloroethane	4-Chloro-3-methylphenol	Pyrene	
1,2,3-Trichloropropane	2-Methylnaphthalene	Butylbenzylphthalate	
Isopropylbenzene	1-Methylnaphthalene	Benz[a]anthracene	
Bromobenzene	Hexachlorocyclopentadiene	Chrysene	
2-Chlorotoluene	2,4,6-Trichlorophenol	bis(2-Ethylhexyl)phthalate	
n-Propylbenzene	2,4,5-Trichlorophenol	Di-n-octylphthalate	
4-Chlorotoluene	Diphenylamine	Benzo[b]fluoranthene	
1,3,5-Trimethylbenzene	Azobenzene	Benzo[k]fluoranthene	
tert-Butylbenzene	2-Chloronaphthalene	Benzo[a]pyrene	
1,2,4-Trimethylbenzene	2-Nitroaniline	Indeno[1,2,3-cd]pyrene	
sec-Butylbenzene	1,4-Dinitrobenzene	Dibenz[a,h]anthracene	
Phenol	Dimethylphthalate	Benzo[g,h,i]perylene	
bis(2-Chloroethyl)ether	1,3-Dinitrobenzene		
Aniline	Acenaphthylene		

Table 5. Target Compounds for Second Tier Chemical Testing, Part B

Pesticides	Polychlorinated Biphenyls				Pharmaceuticals
a-BHC	PCB 2	PCB 73	PCB 81	PCB 158	Ampicillin Trihydrate
b-BHC	PCB 3	PCB 49	PCB 87	PCB 129	Amoxicillin Trihydrate
g-BHC	PCB 4	PCB 47	PCB 115	PCB 178	Oxytocin
d-BHC	PCB 10	PCB 48	PCB 85	PCB 175	Florfenicol
Heptachlor	PCB 7	PCB 75	PCB 136	PCB 187	Ceftiofur
Aldrin	PCB 9	PCB 104	PCB 77	PCB 183	Tilmicosin
Heptachlor epoxide	PCB 6	PCB 35	PCB 110	PCB 128	Furosemide
g-Chlordane	PCB 8	PCB 44	PCB 154	PCB 167	Flunixin meglumine
Endosulfan I	PCB 5	PCB 59	PCB 82	PCB 185	Fenbendazol
a-Chlordane	PCB 19	PCB 37	PCB 151	PCB 174	Doramectin
Dieldrin	PCB 12	PCB 42	PCB 135	PCB 177	Tripelennamine
4,4'-DDE	PCB 13	PCB 71	PCB 144	PCB 202	hydrochloride
Endrin	PCB 18	PCB 41	PCB 124	PCB 171	
Endosulfan II	PCB 17	PCB 64	PCB 147	PCB 156	
4,4'-DDD	PCB 15	PCB 40	PCB 107	PCB 173	
Endrin aldehyde	PCB 24	PCB 103	PCB 123	PCB 157	
Endosulfan sulfate	PCB 27	PCB 67	PCB 149	PCB 201	
4,4'-DDT	PCB 16	PCB 100	PCB 118	PCB 172	
Endrin ketone	PCB 32	PCB 63	PCB 134	PCB 197	
Methoxychlor	PCB 34	PCB 74	PCB 114	PCB 180	
	PCB 29	PCB 70	PCB 131	PCB 193	
	PCB 54	PCB 66	PCB 122	PCB 191	
	PCB 26	PCB 93	PCB 165	PCB 200	
	PCB 25	PCB 95	PCB 146	PCB 170	
	PCB 31	PCB 91	PCB 188	PCB 190	
	PCB 50	PCB 56	PCB 153	PCB 199	
	PCB 28	PCB 60	PCB 132	PCB 196	
	PCB 20	PCB 92	PCB 105	PCB 203	
	PCB 33	PCB 84	PCB 141	PCB 189	
	PCB 53	PCB 90	PCB 179	PCB 208	
	PCB 51	PCB 101	PCB 137	PCB 195	
	PCB 22	PCB 99	PCB 176	PCB 207	
	PCB 45	PCB 119	PCB 130	PCB 194	
	PCB 46	PCB 83	PCB 138	PCB 205	
	PCB 69	PCB 97	PCB 163	PCB 206	
	PCB 52	PCB 117	PCB 164		

Table 6. Summary of Data Collected

Site Type	Gas Type	Number of Samples from former GTI projects	Number of Samples from industrial customers	Number of Samples from literature search	Total number of samples
Dairy Farm	Biogas	12	41	6	59
Dairy Farm	Biogas (Partially Clean)	7	-	-	7
Dairy Farm	Biomethane	23	-	-	23
Landfill	Biogas	14	170	21	205
Landfill	Biogas (Partially Clean)	-	-	2	2
Landfill	Biomethane	16	-		16
Wastewater Treatment Plant	Biogas	5	135	8	148
Wastewater Treatment Plant	Biomethane	5	-	-	5
Natural Gas	Natural Gas	38	20	-	58
Total					523

1.1.3 *Task 2 – Microbial/Chemical Profile in Raw Biogas Pipeline*

1.2.1.1 Microbial profile in raw biogas

GTI collected many DNA samples during the previous Dairy Farm Biogas project, and some of the samples were used in this project to determine the microbial profile in raw biogas samples. There are three sources from which DNA was isolated: 1) directly from biogas filter, 2) from positive MPN culture incubated under aerobic condition, 3) and from positive MPN culture incubated under anaerobic condition.

The filter sample was placed in a 50-ml tube with 30 ml of sterile phosphate buffered saline (PBS, pH 7.2 ± 0.1), votexed for 5-10 sec, and sonicated for 2 min ± 5 sec in waterbath sonicator filled with fresh aqueous solution of 0.3% vol/vol Tween 80. After sonication, the filter suspension was used for Most Probable Number (MPN) test and DNA extraction. The MPN test determines the number of live heterotrophic bacteria in the filter samples carried over from biodegradation process. MPN tests were performed in thioglycolate medium (TG media) in triplicate with serial dilutions of filter suspension samples. After 7 days incubation at 37 ºC aerobically and anaerobically, the positive culture bottles were scored and the number of heterotrophic bacteria determined using a statistically derived table (Most Probable Number from Serial Dilution, Bacteriological Analytical Manual, FDA, February 2006). The positive MPN culture then was used for DNA extraction.

DNA extraction for filter suspension samples without prior growth and positive MPN culture after growth was performed using a FastDNA SPIN Kit for Soil (MP Biomedicals LLC). DNA

was used to determine the microbial profile in filter samples and MPN grown samples. The extracted DNA was amplified with polymerase chain reaction (PCR) using various primers specific to the target bacteria groups. For heterotrophic bacteria, universal primer pair BA8F/UN1492R was used to target 16S rRNA gene, and if it failed, a 2nd universal primer pair BA338F/BA1392R was used for a nested PCR to amplify the target 16S rRNA gene from the samples. For acid-producing bacteria (APB), two pairs of primers were used to amplify the *ackA* (ackA-3F and ackA-4R) and *buk* (buk-5F and buk-6R) genes, respectively. Two pairs of primers (IOB-F486 and IOB-R1132, and Gall-F704 and IOB-R1000) were also used to amplify the 16S rRNA gene from iron-oxidizing bacteria (IOB).

The PCR products were purified using a QIAquick PCR Purification Kit, and the purified PCR products were inserted into the pGEM-T Easy Vector System I (Promega Corp., Madison, Wiscosin). The vectors were then transformed into DH5α Subcloning Efficiency Chemically Competent Cells purchased from Invitrogen (Carlsbad, California), and the cells were inoculated onto LB agar medium for screening of white colonies after overnight incubation at 37 ºC. The white colonies were picked and their DNA prepared for sequencing. The sequences were analyzed with the Blast program in the GenBank database and IDs of heterotrophic bacteria, APB, and IOB were determined.

All 24 heterotrophic bacteria sequences isolated from three filter samples without growing in the culture medium were closely related to *Paenibacillus* sp. (Table 7). However, after the filter suspension samples were grown in culture medium, the profile of dominant heterotrophic bacteria changed to *Bacillus* sp. under aerobic condition (14 out of 24 sequences) and diffident *Paenibacillus* sp. under anaerobic condition (16 out of 16 sequences) (Table 8 and Table 9). The results indicated that the dominant heterotrophic bacteria in raw biogas derived from dairy biomass belong to two genera, i.e. *Paenibacillus* and *Bacillus*.

Table 7. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated directly from 3 Filter Samples without Growth using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Paenibacillus glucanolyticus	AB073189	99	3
Paenibacillus glucanolyticus strain FR1_105	EU373524	99	1
Paenibacillus sp. isolate P14-7	AJ297712	96	1
Paenibacillus sp. JAM-FM32	AB526335	99-100	19

Table 8. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated from 3 Positve Aerobic MPN Cultures using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus licheniformis isolate CCM28B	FN433039	100	1
Bacillus licheniformis strain CICC 10087	GQ375232	100	1
Bacillus licheniformis strain CICC 10181	GQ375235	100	2
Bacillus licheniformis strain NBST2	GU011947	99	1
Bacillus licheniformis strain nju-1411-1	FJ915147	99-100	3
Bacillus licheniformis strain YP1A	EF105377	100	1
Bacillus sp. strain R-30915	AM910273	99	3
Bacillus sp. FE-1	EU271855	99	1
Bacillus sphaericus strain 601	DQ350820	98	1
Bordetella avium 197N	AM167904	98	2
Sporosarcina ginsengisoli	AB245381	96-99	2
Sporosarcina luteola	AB473560	100	1
Uncultured bacterium clone 101-68	EF157238	98	3
Uncultured bacterium clone 2G4-89	EU160423	98	1
Uncultured bacterium clone B1	FJ868757	96	1

Table 9. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated from 2 Positve Anaerobic MPN Cultures using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Paenibacillus barengoltzii strain THWCS9	GQ284356	98-99	2
Paenibacillus barengoltzii strain THWCSN47	GQ284370	98	1
Paenibacillus sp. strain HanTHS1	AM283040	98	2
Paenibacillus sp. 5T01	AM162346	99	4
Paenibacillus sp. enrichment culture clone 9	FJ930068	99-100	7

The attempt to directly amplify ackA and buk genes from filter suspension samples without prior growth in the medium failed; therefore the dominant profile of APB was derived from samples after they were inoculated and grown in the culture medium. *Bacillus licheniformis*, *Geobacillus* sp., and *Clostridium acetobutylicum* were the dominant acid-producing species in raw biogas derived from dairy biomass (Table 10 and Table 11).

Table 10. The Closest Relatives of APB Sequences Isolated from 3 Positve Aerobic MPN Cultures using Primers Targeting ackA and buk Genes

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency (%)
Bacillus licheniformis ATCC 14580	CP000002	93-100	34
Clostridium acetobutylicum ATCC 824	AE001437	74-75	2
Geobacillus sp. WCH70	CP001638	75-76	3
Geobacillus sp. Y412MC10	CP001793	76	1
Methanosarcina mazei strain Goe1	AE008384	73	1

Table 11. The Closest Relatives of APB Sequences Isolated from 2 Positve Anaerobic MPN Cultures using Primers Targeting ackA and buk Genes

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency (%)
Bacillus anthracis str. A0248	CP001598	79-82	9
Bacillus cereus E33L	CP000001	98-100	7
Bacillus pumilus SAFR-032	CP000813	90-92	3
Clostridium acetobutylicum ATCC 824	AE001437	73-77	5
Geobacillus sp. Y412MC10	CP001793	74-75	8
Methanosarcina acetivorans str. C2A	AE010299	75	1
Vibrio fischeri MJ11 chromosome I	CP001139	74	1

Plans for Future Activity

Continue to work on Task 1 and Task 2 work activities and begin work on Task 4 Preliminary MIC Model Development (which will be primarily performed by subcontractor, Southwest Research Institute.

References

1. Angostini, R.A. and R.D. Young, *A case history: Investigations of microbially influenced corrosion in a west Texas waterflood. NACE paper no. 119.* 1990.
2. Farthing, S., *Company combats MIC with aggressive control program.* Pipeline and Gas Industry, 1997. **Oct '97**: p. 43-47.
3. Pope, D.H. and R.M. Pope, *Guide for the Monitoring and Treatment of Microbiologically Influenced Corrosion in the Natural Gas Industry.* 1998, Gas Research Institute.
4. Pope, D.H., et al., *Mitigation strategies for microbially influenced corrosion in gas industry facilities.* Corrosion '89, 1989: p. NACE paper 192.
5. Strickland, L.N., R. T. Fortnum, B. W. DuBose, *A case history of microbiologically influenced corrosion in the Lost Hills oilfield, Kern county California.* Corrosion '96, 1996: p. NACE paper 297.
6. Emde, K.M.E., D. W. Smith, and R. Facey, *Initial investigation of microbially influenced corrosion (MIC) in a low temperature water distribution system.* Water Research, 1992. **26**: p. 169-175.
7. Angell, P., *Understanding microbially influenced corrosion as biofilm-mediated changes in surface chemistry.* Curr. Opin. Biotechnol., 1999. **10**(3): p. 269-72.
8. Dzierzewicz, Z., B. Cwalina, L. Weglarz, S. Glab, *Isolation and evaluation of corrosive aggressivity of wild strains of sulfate reducing bacteria.* Acta Microbiologica Polonica, 1992. **41**: p. 211-221.
9. Dzierzewicz, Z., et al., *The relationship between microbial metabolic activity and biocorrosion of carbon steel.* Res. Microbiol., 1997. **148**(9): p. 785-93.
10. Hamilton, W.A., *Sulphate-reducing bacteria and anaerobic corrosion.* Annu. Rev. Microbiol., 1985. **39**: p. 195-217.
11. Horn, J. and D. Jones, *Microbiologically influenced corrosion: Perspectives and approaches*, in *Manual of environmental microbiology*, C.J. Hurst, Crawford, R. L., Knudsen, G. R., McInerney, M. J., Stetzenbach, L. D., Editor. 2002, ASM Press: Washinton, DC. p. 1072-1083.
12. Zhu, X.Y., *"Detection and Mitigation of Microbiologically-Influenced Corrosion (MIC) in Natural Gas Pipelines". GTI Project 20153, completed in March 2007.* 2007.
13. Zhu, X.Y., H. Modi, and J.J. Kilbane II, *Efficacy and risks of nitrate application for the mitigation of SRB-induced corrosion.* In Proceedings of the NACE International Annual Conference, Corrosion/2006, Paper #06524. San Diego, CA., 2006: p. 1-41.
14. Li, S.Y., et al., *Microbiologically Influenced Corrosion of Carbon Steel Exposed to Anaerobic Soil.* Corrosion, 2001. **57**(9): p. 815-828.
15. Kane, R.D. and S. Campbell, *Real-Time Corrosion Monitoring of Steel Influenced by Microbial Activity (SRB) in Simulated Seawater Injection Environments.* CORROSION NACExpo'04, 2004: p. NACE Paper 04579.

16.   King, R.A. and D.S. Wakerley, *Corrosion of Mild Steel by Ferrous Sulphide.* Br Corros J, 1973. **8**(1): p. 41-45.

17.   King, R.A., J.D.A. Miller, and D.S. Wakerley, *Corrosion of Mild Steel in Cultures of Sulphate-Reducing Bacteria: Effect of changing the soluble iron concentration during growth.* Br Corros J, 1973. **8**(2): p. 89-93.

18.   Jack, T.R., et al., *Corrosion Consequences of Secondary Oxidation of Microbial Corrosion.* Corrosion, 1998. **54**(3): p. 246-252.

19.   Zhu, X.Y., *"Bioreactor/Chemostat System for Rapid Biodemilitarization of Munitions" for DOD/DARPA, subcontracted from SRI. Subcontract No. 41-000810. GTI project No. 20569. Completed in Angust 2008.*

20.   de Franca, F.P. and M.T.S. Lutterbach, *Variation in sessile microflora during biofilm formation on AISI-304 stainless steel coupons.* Journal of industrial microbiology, 1996. **17**(1): p. 6-10.

21.   Pope, D.H. and R.M. Pope, *Microbiologically Influenced Corrosion in Fire Protection Sprinkler Systems*, in *A Practical Manual on Microbiologically Influenced Corrosion*, J.G.S. II, Editor. 2001, NACE International: Houston, TX. p. 5.1-5.7.

22.   NACE, *Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines.* NACE Standard TM0106-2006, 2006.

23.   Batista, J.F., R. F. Pereira, J. M. Lopes, M. F. Carvalho, M. J. Feio, M. A. Reis,, *In situ corrosion control in industrial water systems.* Biodegradation, 2000. **11**(6): p. 441-8.

24.   Kasahara, K. and F. Kajiyama, *Role of Sulfate Reducing Bacteria in the Localized Corrosion of Buried Pipes.* Biologically Induced Corrosion; Proceedings of the International Conference on Biologically Induced Corrosion. June 10-12, 1985, Gaithersburg, Maryland. Houston, TX, NACE., 1986: p. 171-183.

25.   Shi, X., R. Avci, and Z. Lewandowski, *Microbially deposited manganese and iron oxides on passive metals—their chemistry and consequences for material performance.* Corrosion, 2002. **58**: p. 728.

26.   Waters, M.S., et al., *Simultaneous interferometric measurement of corrosive or demineralizing bacteria and their mineral interfaces.* Appl Environ Microbiol., 2009. **75**(5): p. 1445-1449.

27.   Little, B.J., P.A. Wagner, and Z. Lewandowski, *The role of biomineralization in microbiologically influenced corrosion, p. 294/1–294/18. In Proceedings of the CORROSION/98 Research Topical Symposia. NACE International, Houston, TX.* 1998.

28.   Pope, D.H., *Microbiologically Influenced Corrosion of Internal Aspects of Natural Gas Industry Pipelines and Associated Equipment: Mechniasms, Diagnosis, and Mitigation*, in *A Practical Manual on Microbiologically Influenced Corrosion*, J.G.S. II, Editor. 2001, NACE International: Houston, TX. p. 6.13-6.25.

29.   Pope, D.H., et al., *Organic Acid Corrosion of Carbon Steel: A Mechanism of Microbiologically Influenced Corrosion.* In Proceedings of the NACE International Annual Conference, Corrosion/1988, Paper #79. Houston, TX., 1988.

30.   Graves, J.W., E. H. Sullivan, *Internal corrosion in gas gathering systems and transmission lines.* Materials Protection, 1996. **5**: p. 33-37.

31.   Kholodenko, V.P., S. K. Jigletsova, V. A. Chugnov, V. B. Rodin, V. S. Kobelev, S. V. Karpov, *Chemicomicrobiological diagnostics of stress corrosion cracking of trunk pipelines.* Appl. Biochem. Microbiol., 2000. **36**: p. 594-601.

32.   Pope, D.H., T. P. Zintel, B. A. Cookingham, R. G. Morris, D. Howard, R. A. Day, J. R. Frank, and G. E. Pogemiller, *Mitigation strategies for microbially influenced corrosion in gas industry facilities.* Corrosion '89, 1989: p. NACE paper 192.

33.   Pope, D.H., R. M. Pope, *Guide for the monitoring and treatment of microbiologically influenced corrosion in the natural gas industry.* 1998, Gas Research Institute.

34.   Scott, P.J.B., *Expert Consensus on MIC: Prevention and Monitoring.* Materials Performance, 2004(3): p. 2-6.

35.   Jack, T.R., et al., *Microbiologically Influenced Corrosion Testing*, in *The Characterization of Sulfate-Reducing Bacteria in Heavy Oil Waterflood Operations*, J.R. Kearns and B.J. Little, Editors. 1994, Philadelphia, PA: ASTM. p. 108.

36.   von Wolzogen Kuhr, C.A.H. and L.S. van der Vlugt, *The graphitization of cast iron as an electrochemical process in anaerobic soils.* Water Research, 1934. **18**: p. 147-165.

37.   McNeil, M.B. and B.J. Little, *Mackinawite Formation During Microbial Corrosion,.* Corrosion, 1990. **46**: p. 599-600.

38.   Tributsch, H., et al., *Role of Transient Iron Sulfide Films in Microbial Corrosion of Steel.* Corrosion, 1998. **54**(3): p. 216-227.

39.   Hardy, J.A. and J.L. Brown, *The Corrosion of Mild Steel By Biogenic Sulfide Films Exposed to Air.* Corrosion, 1984. **40**(12): p. 650.

40.   Jack, T.R. and M.J. Wilmott, *Indicator Minerals Formed During External Corrosion of Line Pipe.* MP, 1995. **34**: p. 19.

41.   Pope, D.H. and E.A.M. III, *Some Experiences With Microbiologically Influenced Corrosion of Pipelines.* MP, 1995. **34**(5): p. 23.

42.   Gibson, G.R. and X. Wang, *Regulatory effects of bifidobacteria on the growth of other colonic bacteria.* J. Appl. Bacteriol., 1994. **77**(4): p. 412-20.

43.   Zellner, G., et al., *Anaerofilum pentosovorans gen. nov., sp. nov., and Anaerofilum agile sp. nov., two new, strictly anaerobic, mesophilic, acidogenic bacteria from anaerobic bioreactors.* Int. J. Syst. Bacteriol., 1996. **46**(4): p. 871-5.

44.   Kanauchi, O., et al., *Increased growth of Bifidobacterium and Eubacterium by germinated barley foodstuff, accompanied by enhanced butyrate production in healthy volunteers.* Int. J. Mol. Med., 1999. **3**(2): p. 175-9.

45.   Broda, D.M., et al., *Clostridium algidixylanolyticum sp. nov., a psychrotolerant, xylan-degrading, spore-forming bacterium.* Int. J. Syst. Evol. Microbiol., 2000. **50 Pt 2**: p. 623-31.

46.   Vetting, M.W., et al., *Structure of Acinetobacter strain ADP1 protocatechuate 3, 4-dioxygenase at 2.2 A resolution: implications for the mechanism of an intradiol dioxygenase.* Biochemistry, 2000. **39**(27): p. 7943-55.

47.   Zhu, X.Y., J. Lubeck, and J.J. Kilbane, 2nd, *Characterization of microbial communities in gas industry pipelines.* Appl Environ Microbiol, 2003. **69**(9): p. 5354-63.

48.   Little, B.J. and P. Wagner, *Microbiologically Influenced Corrosion*, in *Peabody's Control of Pipeline Corrosion*, R.L. Bianchetti, Editor. 2001, Houston, TX: NACE, 2001.

49.   Towers, R., *Accelerated Corrosion in Cargo Tanks of Large, Double-Hull Ships, Causes and Countermeasures.* Protective Coatings Europe, 2000(3): p. 30-42.

50.   Hines, M.E., P.T. Visscher, and R. Devereux, *Sulfur cycling*, in *Manual of Environmental Microbiology*, L.D. Stetzenbach, Editor. 2002, ASM Press: Washington, DC. p. 427-438.

51.   Mudryk, Z.J., et al., *The occurrence and activity of sulphate-reducing bacteria in the bottom sediments of the Gulf of Gdañsk.* OCEANOLOGIA, 2000. **42**(1): p. 105-117.

52. Caumette, P., *Ecology and physiology of phototrophic bacteria and sulphate-reducing bacteria in marine salterns.* Experientia, 1993. **49**: p. 473-481.

53. Bak, F. and P. N., *Sulfate-reducing bacteria in littoral sediment of Lake Constance.* FEMS Microbiol. Ecol., 1991. **85**: p. 43–52.

54. Le Borgne, S., et al., *Detecting and Monitoring Bacteria in Seawater Injection Systems.* Materials Performance, 2007. **46**(11): p. 52-56.

55. Jack, T.R., *MIC in Underground Environments: External Corrosion in the Gas pipeline Industry*, in *A Practical Manual on Microbiologically Influenced Corrosion*, J.G.S. II, Editor. 2001, NACE International: Houston, TX. p. 6.1-6.42.

56. Campaignolle, X., et al., *Stabilization of Localized Corrosion of Carbon Steel by Sulfate-Reducing Bacteria.* Nace International Corrosion 93, Paper no. 302, 1993: p. 1-7.

57. Daumas, S., M. Magot, and J.L. Crolet, *Measurement of the net production of acidity by a sulphate-reducing bacterium: experimental checking of theoretical models of microbially influenced corrosion.* Res. Microbiol., 1993. **144**(4): p. 327-332.

58. Hemmingsen, T., H. Vangdal, and T. Våland, *Formation of Ferrous Sulfide Film from Sulfite on Steel Under Anaerobic Conditions.* Corrosion, 1992. **48**(6): p. 475-481.

59. Campaignolle, X. and J.-L. Crolet, *Method for Studying Stabilization of Localized Corrosion on Carbon Steel by Sulfate-Reducing Bacteria.* Corrosion, 1997. **53**(6).

60. Tanji, Y., et al., *Chemical Analysis of an Artificial Biofilm that Enhances or Inhibits Carbon Steel Corrosion.* Corrosion, 2002. **58**(3): p. 232-239.

61. King, L.D. and J.D.A. Miller, *Corrosion by the sulfate reducing bacteria.* Nature, 1971. **233**: p. 491-492.

62. King, R.A., J.D.A. Miller, and J.S. Smith, *Corrosion of mild steel by iron sulfides.* Brit Corrosion J, 1973. **8**: p. 137-142.

63. Angeles-Ch, C., et al., *Microbiologically Influenced Corrosion by Citrobacter in Sour Gas Pipelines.* Materials Performance, 2002. **41**(8): p. 50-55.

64. Little, B.J., R.I. Ray, and R.K. Pope, *Relationship Between Corrosion and the Biological Sulfur Cycle: A Review.* Corrosion, 2000. **56**(4): p. 433.

65. Gonzalez, J.L., et al., *A damage model for assessing pipeline safety in corrosion environments.* Corrosion, 1997. **53**: p. 935-943.

66. Zhu, X.Y., *Rapid Quantification of Butyric Acid-Producing Bacteria Using Real-Time PCR*. 2007, Gas Technology Institute: USA.

67. Zhu, X.Y., *Rapid Quantification of Acetic Acid-Producing Bacteria Using Real-Time PCR*. 2008, Gas Technology Institute: USA.

68. Emerson, D. and W.C. Ghiorse *Isolation, Cultural Maintenance, and Taxonomy of a Sheath-Forming Strain of Leptothrix discophora and Characterization of Manganese-Oxidizing Activity Associated with the Sheath.* Appl. Environ. Microbiol., 1992. **58**: p. 4001-4010.

69. Hanert, H.H., *The genus Siderocapsa (and other iron- or manganese-oxidizing eubacteria*, in *The Prokaryotes, a Handbook on Habitats, Isolation and Identification of Bacteria*, M. Starr, et al., Editors. 1981, Springer-Verlag: New York. p. 1049-1059.

70. Dickinson, W.H. and Z. Lewandowski, *Manganese Biofouling and the Corrosion Behavior of Stainless Steel.* Biofouling, 1996. **10**: p. 79-93.

71. Hamilton, W.A., *Sulphate-reducing bacteria and anaerobic corrosion.* Ann. Rev. Microbiol., 1985. **39**: p. 195-217.

72.   Zhu, X.Y., et al., *Rapid detection and quantification of microbes related to microbiologically influenced corrosion using quantitative polymerase chain reaction.* Corrosion, 2006. **62**: p. 950-955.

73.   Zhu, X.Y., et al., *Application of Quantitative, Real-Time PCR in Monitoring Microbiologically-Influenced Corrosion (MIC) in Gas Pipelines.* In Proceedings of the NACE International Annual Conference, Corrosion/2005, Paper #05493. Houston, TX., 2005: p. 1-20.

74.   Myers, C. and K.H. Nealson, *Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor.* Science, 1988. **240**: p. 1319.

75.   Dubey, R.S. and Upadhyay, *Microbial Corrosion Monitoring by an Amperometric Microbial Biosensor Developed using Whole Cell of Pseudomonas sp.* Biosensors and Bioelectronics, 2001. **16**: p. 995-1000.

76.   Nordstrom, D.K. and G. Southam, *Geomicrobiology of Sulfide Mineral Oxidation*, in *Reviews in Mineralogy Geomicrobiology: Interactions Between Microbes and Minerals*, J.F. Banfield and K.H. Nealson, Editors. 1997, Washington, DC: Mineralogical Society of America. p. 361-390.

77.   Boivin, J., et al., *The Influence of Enzyme Systems on MIC.* CORROSION/90, paper no. 128 (Houston, TX: NACE, 1990). 1990.

78.   Braker, G., A. Fesefeldt, and K.-P. Witzel, *Development of PCR Primer Systems for Amplification of Nitrite Reductase Genes (nirK and nirS) To Detect Denitrifying Bacteria in Environmental Samples.* Appl. Environ. Microbiol., 1998. **64**(10): p. 3769-3775.

79.   Nemati, M., G.E. Jenneman, and G. Voordouw, *Impact of nitrate-mediated microbial control of souring in oil reservoirs on the extent of corrosion.* Biotechnol. Prog., 2001. **17**(5): p. 852-9.

80.   Zintel, T.P., G.J. Licina, and T.R. Jack, *Techniques for MIC Monitoring*, in *A Practical Manual on Microbiologically Influenced Corrosion*, J.G.S. II, Editor. 2001, NACE International: Houston, TX. p. 10.1-10.19.

81.   NACE, *Field Monitoring of Bacterial Growth in Oilfield and Gas Systems.* NACE Standard TM0194-2004.

82.   Little, B., P. Wager, and F. Mansfeld, *Microbiologically Influenced Corrosion of Metals and Alloys.* Int. Mat. Rev., 1991. **36**(6): p. 253-272.

83.   Hernández-Gayosso, M.J., et al., *Microbial consortium influence upon steel corrosion rate, using the electrochemical impedance spectroscopy technique.* Materials and Corrosion, 2004. **55**(9): p. 676-683.

84.   API, *Recommended Practice for Biological Analysis of Subsurface Injection Waters, American Petroleum Institute, Washington, D.C.,.* 1982.

85.   Williams, W.A., J.H. Lobos, and W.E. Cheetham, *A phylogenetic analysis of aerobic polychlorinated biphenyl-degrading bacteria.* Int. J. Syst. Bacteriol., 1997. **47**(1): p. 207-10.

86.   Maidak, B.L., et al., *The RDP-II (Ribosomal Database Project).* Nucleic Acids Res, 2001. **29**(1): p. 173-4.

87.   Muyzer, G., E.C. de Waal, and A.G. Uitterlinden, *Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.* Appl Environ Microbiol, 1993. **59**(3): p. 695-700.

88. Zhu, X.Y., T. Zhong, Y. Pandya, R. D. Joerger, *16S rRNA-based analysis of microbiota from the cecum of broiler chickens.* Appl. Environ. Microbiol., 2002. **68**(1): p. 124-37.

89. Osburne, M.S., T. H. Griossman, P. R. August, I. A. MacNeil, *Tapping into microbial diversity for natural products drug discovery.* ASM News, 2000. **66**: p. 411-417.

90. Torsvik, V., J. Goksoyr, and F.L. Daae, *High diversity in DNA of soil bacteria.* Appl Environ Microbiol, 1990. **56**(3): p. 782-7.

91. Harmsen, H.J., et al., *Population dynamics of propionate-oxidizing bacteria under methanogenic and sulfidogenic conditions in anaerobic granular sludge.* Appl. Environ. Microbiol., 1996. **62**(6): p. 2163-8.

92. Zhu, X.Y. and R.D. Joerger, *Composition of microbiota in content and mucus from cecae of broiler chickens as measured by fluorescent in situ hybridization with group-specific, 16S rRNA-targeted oligonucleotide probes.* Poult Sci, 2003. **82**(8): p. 1242-9.

93. Voordouw, G., *Reverse Sample Genome Probing of Microbial Community Dynamics.* ASM News, 1998. **64**: p. 627-633.

94. Voordouw, G., et al., *Application of Reverse Sample Genoma Probing to the Identification of Sulfate-Reducing Bacteria*, in *Microbiologically Influenced Corrosion Testing, ASTM STP 1232*, J.R. Kearns and B.J. Little, Editors. 1994, Philadelphia, PA: ASTM. p. 188-199.

95. Telang, A.J., S. Ebert, J. M. Foght, D. W. S. Westlake, G. E. Jenneman, D. Gevertz, G. Voordouw, *Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing.* Appl. Environ. Microbiol., 1997. **63**(5): p. 1785-1793.

96. Skovhus, T.L., et al., *Real-Time Quantitative PCR for Assessment of Abundance of Pseudoalteromonas Species in Marine Samples.* Appl. Environ. Microbiol., 2004. **70**(4): p. 2373-2382.

97. Stults, J.R., O. Snoeyenbos-West, B. Methe, D. R. Lovley, D. P. Chandler, *Application of the 5' fluorogenic exonuclease assay (TaqMan) for quantitative ribosomal DNA and rRNA analysis in sediments.* Appl. Environ. Microbiol., 2001. **67**(6): p. 2781-2789.

98. Suzuki, M.T., L.T. Taylor, and E.F. DeLong, *Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 5'-Nuclease Assays.* Appl. Environ. Microbiol., 2000. **66**(11): p. 4605-4614.

99. Brinkman, N.E., et al., *Evaluation of a Rapid, Quantitative Real-Time PCR Method for Enumeration of Pathogenic Candida Cells in Water.* Appl. Environ. Microbiol., 2003. **69**(3): p. 1775-1782.

100. Guy, R.A., et al., *Real-Time PCR for Quantification of Giardia and Cryptosporidium in Environmental Water Samples and Sewage.* Appl. Environ. Microbiol., 2003. **69**(9): p. 5178-5185.

101. Ibekwe, A.M., et al., *Multiplex Fluorogenic Real-Time PCR for Detection and Quantification of Escherichia coli O157:H7 in Dairy Wastewater Wetlands.* Appl. Environ. Microbiol., 2002. **68**(10): p. 4853-4862.

102. Blackburn, F.E., *Non-Bioassay Techniques for Monitoring SRB.* CORROSION/2004 (Houston, TX: NACE, 2004).

103. Dorsey, M.H., et al., *Monitoring for Corrosion and Microbiological Activity in a Cooling Water System.* Plant Power Chemistry, 2002. **4**(12): p. 721-731.

104. Royer, R.A. and R.F. Unz, *Use of Electrical Resistance Probes for Studying Microbiologically Influenced Corrosion.* Corrosion, 2002. **58**(10): p. 863-870.

105. Pope, D.H., *State-of-the-Art Report on Monitoring, Prevention and Mitigation of Microbiolwically lnfluenced Corrosion in the Natural Gas Industrv.* Topical Report, GRI-9210382. Gas Research Institute, Chicago, IL, 1992.

106. Lockwood, S.F., et al., *Microbiologically lnfluenced Corrosion in the Natural Gas Industry.* Topical Report (Unpublished) to Gas Research Institute, February 1993., 1993.

107. Lutey, R.W., *Treatment for the Mitigation of MIC*, in *A Practical Manual on Microbiologically Influenced Corrosion*, J.G. Stoecker, Editor. 1993, Houston, TX: NACE. p. 9.1-9.9.30.

108. Nichols, W.W., et al., *The penetration of antibiotics into aggregates of mucoid and non-mucoid Pseudomonas aeruginose.* J. Gen. Microbiol., 1989. **35**(35): p. 1219-1303.

109. Kajdasz, R.E., R.V. Einstman, and L. Young-Bandala, *Biocidal Efficacy with Respect to Sessile and Planktonic.* IWV, 1984: p. 85-93.

110. McCoy, W.F., *Strategies for the treatment of biological biofouling.*, in *Biological fouling of industrial water systems - A problem solving approach*, M.W. Mittelman and G.G. Geysey, Editors. 1987, Water Micro. Associates: San Diego, CA. p. 247-268.

111. Pope, D.H. and R. Skultety, *Microbiologically lnfluenced Corrosion in Natural Gas Storage Fields: Diagnosis, Monitoring and Control.* NACE International Conference on Microbiologically lnfluenced Corrosion, Paper No. 57. New Orleans, LA., 1995.

112. Webster, B.J. and R.C. Newman, *ASTM STP 1232: Producing Rapid Sulfate-Reducing Bacteria-Influenced Corrosion in the Laboratory*, in *Microbiologically Influenced Corrosion Testing*, J. Kearns and B.J. Little, Editors. 1994, ASTM: West Conshohocken, PA. p. 28-41.

113. Little, B. and R. Ray, *A Perspective on Corrosion Inhibition by Biofilms.* Corrosion, 2002. **58**(5): p. 424-428.

114. Jayaraman, A., et al., *Axenic aerobic biofilms inhibit corrosion of copper and aluminum.* Appl Microbiol Biotechnol., 1999. **52**(6): p. 787-790.

115. Jayaraman, A., et al., *Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ.* Appl Microbiol Biotechnol., 1999. **52**(2): p. 267-275.

116. Zuo, R., *Biofilms: strategies for metal corrosion inhibition employing microorganisms.* Appl Microbiol Biotechnol., 2007. **76**(6): p. 1245.

117. Zuo, R. and T.K. Wood, *Inhibiting mild steel corrosion from sulfate-reducing and iron-oxidizing bacteria using gramicidin-S-producing biofilms.* Appl Microbiol Biotechnol., 2004. **65**(6): p. 747-753.

118. Zuo, R., et al., *Inhibiting mild steel corrosion from sulfate-reducing bacteria using antimicrobial-producing biofilms in Three-Mile-Island process water.* Appl Microbiol Biotechnol., 2004. **64**(2): p. 275-283.

119. Chan, K.Y., L.C. Xu, and H.H. Fang, *Anaerobic electrochemical corrosion of mild steel in the presence of extracellular polymeric substances produced by a culture enriched in sulfate-reducing bacteria.* Environ Sci Technol., 2002. **36**(8): p. 1720-1727.

120. Hernandez, G., et al., *Corrosion Inhibition of Steel by Bacteria.* Corrosion, 1994. **50**(08): p. 603-608.

121. Jayaraman, A., et al., *Axenic Aerobic Biofilms Inhibit Corrosion of SAE 1018 Steel through Oxygen Depletion.* Applied Microbiology and Biotechnology, 1997. **48**: p. 11-17.

122. McCafferty, E. and J.V. McArdle. *Corrosion Inhibition by Biological Siderophore*. in *182nd Society Meeting (Toronto, Canada: The Electrochemical Society)*. 1992.
123. Eashwar, M. and S. Maruthamuthu, *Mechanism of biologically produced ennoblement: Ecological perspectives and a hypothetical model.* Biofouling, 1995. **8**(3): p. 203-213.
124. Little, B., P. Wagner, and F. Mansfeld, *Microbiologically Influenced Corrosion of Metals and Alloys.* Int. Mat. Rev., 1991. **36**(6): p. 253-272.
125. Geesey, G.G., in *Biofouling and biocorrosion in industrial water systems*, H.-C. Flemming and G.G. Geesey, Editors. 1991, Springer-Verlag: Berlin.
126. Heitz, E., in *Microbially influenced corrosion of materials*, E. Heitz, H.-C. Flemming, and W. Sand, Editors. 1996, Springer-Verlag: Berlin, New York.